Articles

Ganglioside G_{M1} and Asialo-G_{M1} at Low Concentration Are Preferentially Incorporated into the Gel Phase in Two-Component, Two-Phase Phosphatidylcholine Bilayers†

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ABSTRACT: Multilamellar liposomes composed of 1:1 dielaidoylphosphatidylcholine:dipalmitoylphosphatidylcholine at 20 °C contain laterally separated gel and liquid-crystalline phases that can be identified by electron microscopy in freeze–etch replicas on the basis of their distinctive morphology. Visualization of marker proteins that specifically bind to glycosphingolipids included in these liposomes has revealed that, at 1 mol % or less, the ganglioside G_{M1} and the neutral asialo-G_{M1} derived from it are localized within the gel-phase regions exclusively. Increasing the mole fraction of the glycosphingolipids results in the appearance of marker in the fluid-phase regions. Another neutral glycosphingolipid, Forssman, does not display a phase preference and is found in both phases at a low mole percent. The phase preference of these three glycosphingolipids depends primarily upon interactions between the hydrophobic moieties of these molecules and the matrix phosphatidylcholines.

Biological membranes are composed of a complex mixture of phospholipids, cholesterol, and glycolipids, organized as a lipid bilayer. Associated with the bilayer are integral and peripheral protein components. Glycosphingolipids, ubiquitous components of mammalian plasma membranes, are localized exclusively in the outer leaflet of the membrane bilayer (Gahmberg & Hakomori, 1973; Steck & Dawson, 1974). Characteristically this leaflet contains a heterogeneous mixture of glycosphingolipids that vary in the composition of the hydrophobic moiety of the molecule anchored in the phospholipid bilayer and in the oligosaccharide portion, which extends into the aqueous environment surrounding the cell. Glycosphingolipids also vary in charge and in the length and degree of unsaturation and hydroxylation of the sphingosine base and amide-linked fatty acid moieties [for reviews, see Thompson and Tillack (1985) and Curatolo (1987)].

Our laboratory has been investigating the organization of glycosphingolipids in lipid bilayers as an approach to understanding their functional role in membranes. We have used liposomal systems in which glycosphingolipids are inserted into phospholipid bilayers of defined composition. Thus far, our studies have employed liposomes consisting of only one phospholipid species into which a single species of glycosphingolipid has been incorporated as a minor component. Morphological techniques, including freeze–etch electron microscopy, have been used to study the distribution of glycosphingolipids in liposomes, taking advantage of the antigenicity of the glycosphingolipid carbohydrate moieties to which antibodies or lectins can be directed. Visualization of the antibody or lectin molecules has been achieved by employing ultrastructural markers such as ferritin or colloidal gold or by visualizing antibodies, Fab fragments, or cholera toxin molecules directly on bilayer surfaces with techniques such as ultrafast freezing and freeze–etch electron microscopy (Tillack et al., 1982; Thompson et al., 1985). We have been especially interested in determining whether glycosphingolipids form compositional domains in membranes or whether they are molecularly dispersed in the phospholipid matrix. These studies have indicated that the charged glycosphingolipid G_{M1} and the neutral Forssman glycosphingolipid are molecularly dispersed in phospholipid bilayers whereas the neutral glycosphingolipid asialo-G_{M1} is present in microdomains of about 16 molecules (Thompson et al., 1985; Rock et al., 1988, 1990; Tillack et al., 1988).

In order to simulate more closely the situation in biological membranes where the membrane contains more than one species of phospholipid, we have used multilamellar liposomes composed of 1:1 dielaidoylphosphatidylcholine (DEPC)[1]:dipalmitoylphosphatidylcholine (DPPC), which exhibit laterally separated fluid- and gel-phase regions, into which minor amounts of different glycosphingolipids have been incorporated. The DEPC–DPPC system was originally described by Grant et al. (1974), who demonstrated that the laterally separated fluid- and gel-phase regions can be identified in freeze–etch replicas by their characteristic morphological appearance. Their studies led to a phase diagram for this two-component system, and they showed that integral membrane proteins preferentially partition into fluid-phase regions. In the studies reported here, the charged ganglioside G_{M1} and the neutral glycosphingolipids asialo-G_{M1} and Forssman were incorporated into 1:1 DEPC:DPPC liposomes labeled with cholera toxin (specific for G_{M1}) or Fab fragments of antibodies (specific for asialo-G_{M1} and Forssman glycolipid). Liposomes were then fast frozen in a temperature- and humidity-con-

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[1] Abbreviations: DEPC, dielaidoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine.

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trolled environmental chamber and examined by freeze–etch electron microscopy. The distribution of labels specific for the glycosphingolipids was evaluated on freeze–etch replicas, and the distribution of the label over gel- and fluid-phase regions of the phospholipid bilayer was determined. It is concluded that the charged ganglioside G_{M1} and its neutral derivative asialo-G_{M1} distribute preferentially into gel-phase regions in mixed gel–fluid-phase liposomes, whereas Forssman glycolipid distributes evenly in the two phases.

Materials and Methods

DEPC, DPPC, 1-palmitoyl-2-oleoylphosphatidylcholine (POPC),[1] and distearoylphosphatidylcholine (DSPC)[1] were obtained from Avanti Polar Lipids, Inc., Pelham, AL, and used without further purification. Forssman glycolipid was isolated from canine small intestine by the method of Sung et al. (1973). G_{M1} was purchased from Sigma Chemical Co., St. Louis, MO. Asialo-G_{M1} was prepared from G_{M1} by formic acid desialation (Tillack et al., 1982). Glycosphingolipid fatty acid and sphingolipid base compositions were determined by the combined gas chromatography/mass spectroscopy method of Aaronson and Martin (1983).

Multilamellar liposomes were prepared by spraying a chloroform solution of the mixed lipid onto a glass plate as described earlier (Thompson et al., 1985). Liposomes were hydrated in a pH 7.4 buffer containing 10 mM sodium phosphate and 10 mM NaCl at least 10 °C above the transition temperature of the matrix lipid, usually to a concentration of 5 mM total lipid.

Cholera toxin was obtained from Sigma and dialyzed against 10 mM Tris buffer containing 10 mM NaCl, pH 7.4. Fab fragments were prepared from rabbit polyclonal anti-Forssman and anti-asialo-G_{M1} IgG by the method of Coulter and Harris (1983) and dialyzed against 10 mM phosphate/10 mM NaCl buffer, pH 7.4.

For labeling experiments, 40 μL of liposomes (200 nmol of lipid phosphorus) was mixed with 150 μL of a solution of IgG (1.8 mg/mL) or Fab (0.8 mg/mL) and incubated at the appropriate temperature for 1–2 h. Liposomes containing G_{M1} were labeled with cholera toxin, as previously described (Thompson et al., 1985). Labeled liposomes were pelleted briefly (1 min at 3000*g*) in a microfuge.

The pellet of labeled liposomes was resuspended in 20 μL of buffer. A 10-μL portion of this suspension was loaded onto a 3 mm^2 by 0.8 mm thick piece of fixed, washed rabbit lung and placed on the sample head of a rapid freezing apparatus (Heuser, 1983). The cryopress was located in a temperature- and humidity-controlled environmental room to allow cryofixation above or below the T_m of the matrix phospholipid. Humidification of the room was necessary to prevent lowering of the temperature of the specimen prior to freezing due to evaporational cooling. The sample was frozen by slamming it onto a liquid helium cooled copper or silver block and then immediately stored under liquid nitrogen. Freeze–etching and platinum/carbon rotary shadowing were performed as described previously with a Balzers BAF-300 freeze–etching apparatus (Thompson et al., 1985). Replicas were examined with a Zeiss 902 electron microscope.

Results

Distribution of Charged Glycosphingolipids in Mixed Fluid–Gel-Phase Liposomes. Liposomes containing a 1:1 mixture of DEPC–DPPC at 20 °C show lateral separation of a gel phase rich in DPPC from a liquid-crystalline phase rich in DEPC, as originally reported by Grant et al. (1974). Freeze–etch electron microscopy of these liposomes shows a gel phase characterized by the presence of $P_{\beta'}$ ripples and a fluid phase that has a smooth appearance, the smooth and rippled areas being about equal in proportion (Figure 1A). The phase diagram for this phospholipid mixture, shown in Figure 2, is adapted from that reported by Grant et al. (1974).

Figure 1B shows a liposome composed of a 1:1 mixture of DEPC–DPPC containing 1 mol % G_{M1} that has been labeled with cholera toxin and quenched from 20 °C. The cholera toxin is almost exclusively localized over ridged (gel-phase) regions of the liposome. Cholera toxin is a specific marker for the ganglioside G_{M1} and appears as a distinct 90-Å particle on freeze–etch replicas (Thompson et al., 1985). Incorporation of this small amount of G_{M1} did not appear to alter the proportion of ridged and smooth areas that were visualized in the liposomes.

As the mole fraction of G_{M1} in 1:1 DEPC–DPPC mixtures at 20 °C is increased beyond 1 mol %, the amount of cholera toxin appearing in the smooth (fluid-phase) regions of the liposome increases, as seen in Figure 3. A 1:1 DEPC:DPPC liposome containing 2.8 mol % G_{M1} that has been labeled with cholera toxin and quenched from 20 °C is shown in Figure 1C. The label is closely packed over the ridged (gel-phase) regions but is also present on the smooth (fluid-phase) regions. Full coverage of both ridged and smooth areas occurs when 3.2 mol % G_{M1} is incorporated into this system.

In order to simulate more closely the situation in biological membranes in which glycosphingolipids are localized to the external leaflet of the membrane, we prepared 1:1 DEPC:DPPC liposomes and incubated then with an aqueous dispersion of G_{M1} micelles at 50 °C, which results in the incorporation of G_{M1} only into the outer leaflets of the external bilayer of the liposomes (Felgner et al., 1981). Labeling of these compositionally asymmetric liposomes with cholera toxin at 20 °C gave the same label distribution that we observe with liposomes prepared as described previously in which G_{M1} is distributed in both leaflets of the bilayer. Figure 1D shows that the cholera toxin is again predominantly located over the ridged (gel-phase) areas of the liposome.

The effect of temperature on the distribution of G_{M1} in 1:1 DEPC:DPPC liposomes was investigated. At 38 °C, a 1:1 mixture of DEPC–DPPC is in the fluid phase as shown by the phase diagram in Figure 2. In accordance with this, these liposomes containing 1 mol % G_{M1} show only a smooth appearance with no ridged areas when examined by freeze–etch electron microscopy. Cholera toxin is distributed over the entire outer surface of the liposome and is in a slightly clustered arrangement (Figure 4A). At 4 °C, cholera toxin is uniformly distributed over the entire surface of 1:1 DEPC:DPPC liposomes, as shown in Figure 4B. At 4 °C, the phase diagram predicts an all-gel phase and the freeze–fractured liposomes show no $P_{\beta'}$ rippling. At the same concentration of G_{M1}, there is more label present per unit area on the liposomes quenched from 4 °C than on those quenched from 38 °C.

Elaidic acid, the trans analogue of oleic acid, and DEPC are not found in living organisms except in trace amounts. To eliminate the possibility that G_{M1} interacts unfavorably with DEPC, G_{M1} was incorporated into pure DEPC liposomes and labeled with cholera toxin at 4, 20, and 38 °C. Freeze–etch studies showed the cholera toxin label over the entire liposomal surface at all three temperatures, indicating that G_{M1} is able to insert into pure DEPC bilayers in both fluid and gel phases (data not shown).

In order to ascertain whether the $P_{\beta'}$ segregation of G_{M1} at low concentration is specific to the DEPC–DPPC system, we examined equimolar mixtures of POPC with DPPC and

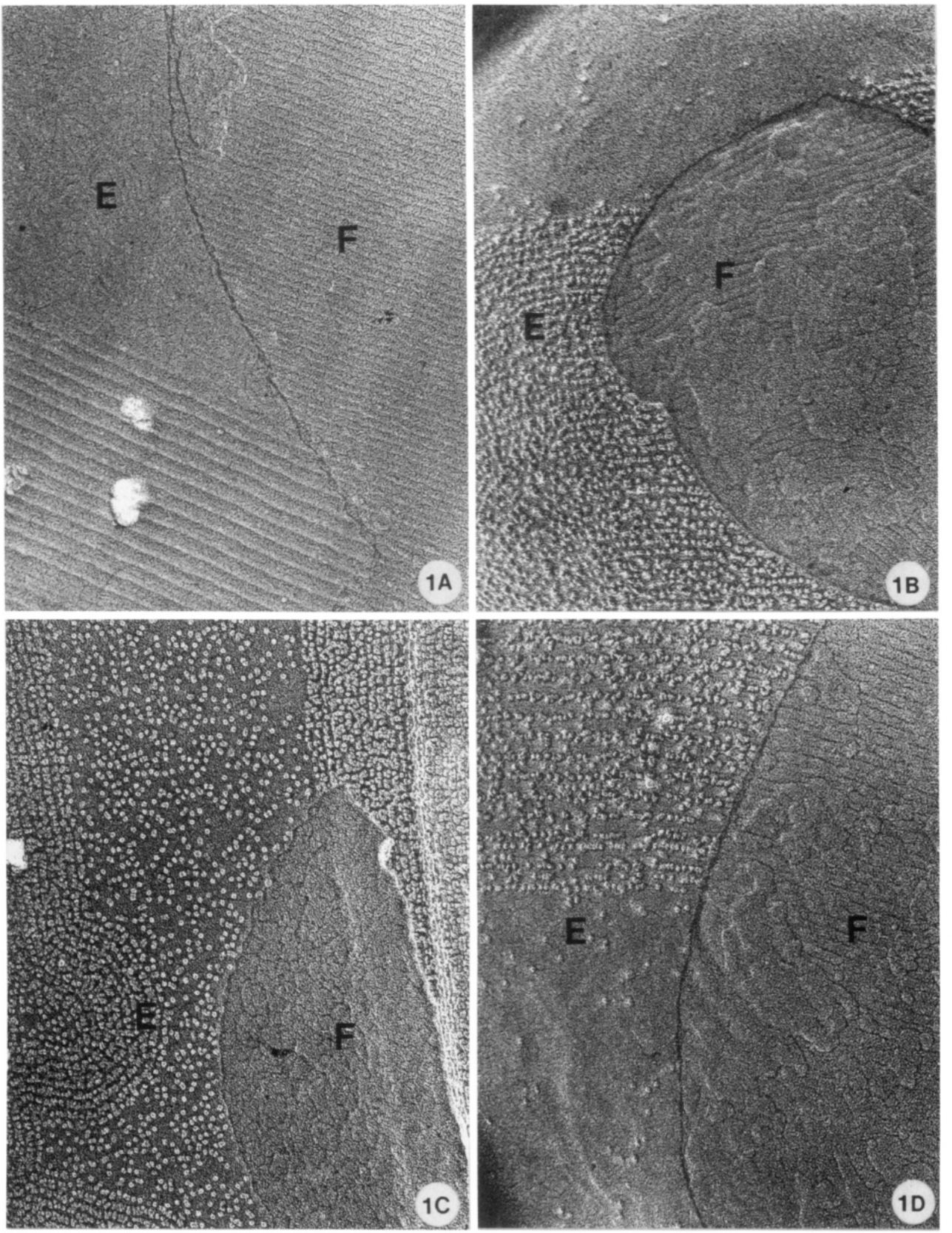

FIGURE 1: Freeze–etch electron micrographs of 1:1 DEPC:DPPC liposomes containing varying amounts of G_{M1} quenched from 20 °C: (A) liposome containing 0 mol % G_{M1} showing lateral separation of the gel phase (rippled area) and liquid-crystalline phase (smooth area) on the external fracture face (E); (B) liposome containing 1.0 mol % G_{M1} labeled with cholera toxin; (C) liposome containing 2.8 mol % G_{M1} labeled with cholera toxin; (D) liposome containing approximately 1.0 mol % G_{M1} asymmetrically incorporated into the outer monolayer and labeled with cholera toxin. F = bilayer fracture face. Magnification is 130000×.

DMPC with DSPC. At 20 °C the equimolar mixture of POPC–DPPC showed the existence of both smooth and rippled phases, which we identify with fluid and gel phases, respectively. The phase diagram for this system determined by differential scanning calorimetry shows there to be roughly equal amounts of coexisting fluid and gel phases at this com-

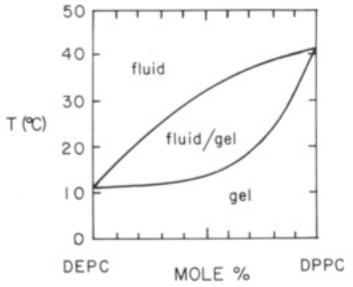

FIGURE 2: Phase diagram for DEPC:DPPC mixtures. Data adapted from Grant et al. (1974).

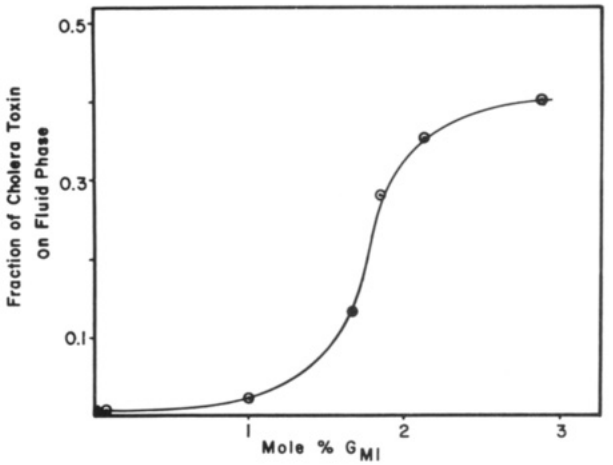

FIGURE 3: Fraction of cholera toxin label on the fluid phase of 1:1 DEPC:DPPC liposomes as a function of liposomal G_{M1} concentration. Below 1.0 mol % G_{M1}, the label is predominantly on the gel-phase regions.

position and temperature (Davis et al., 1980). Liposomes formed from a 1:1 POPC–DPPC mixture containing 1.4 mol % G_{M1}, when labeled with cholera toxin and quenched from 20 °C, displayed a marked preference of cholera toxin for the rippled areas (Figure 5A). A similar result was obtained with liposomes formed from 1:1 DMPC–DSPC mixtures containing 0.03 mol % G_{M1} at 35 °C (Figure 5B). The phase diagram for this system shows that roughly equal amounts of fluid and gel phases are present at 35 °C at this composition (Knoll et al., 1981).

Distribution of Neutral Glycosphingolipids in Mixed Fluid–Gel-Phase Multilamellar Liposomes. The neutral glycosphingolipid asialo-G_{M1} was prepared by formic acid hydrolysis from the same G_{M1} preparation used in the previous studies. Thus, the only difference between the two preparations is the lack of one sialic acid moiety in the oligosaccharide chain of the glycosphingolipid. Liposomes prepared from 1:1 mixtures of DEPC–DPPC with 1 mol % asialo-G_{M1} were labeled with Fab fragments prepared from anti-asialo-G_{M1} antibody molecules. When this preparation was quenched from 20 °C and then examined by freeze–etch electron microscopy, the Fab was localized predominantly over the ridged (gel-phase) regions of the liposomes, as shown in Figure 6A. Thus, the charged sialic acid moiety does not appear to be the determining factor in the preference of G_{M1} for the gel phase, but rather the interaction of the hydrophobic moiety of the glycosphingolipid molecule with the phospholipid matrix appears to be the more important factor.

Forssman antigen, a neutral pentasaccharide-bearing glycosphingolipid, was incorporated into 1:1 DEPC:DPPC liposomes and labeled with Fab fragments prepared from anti-Forssman antibody molecules. Figure 6B shows such a liposome containing 1 mol % Forssman quenched from 20 °C, and in this system the Fab label appears evenly distributed over the ridged (gel) and smooth (fluid) regions of the liposome. Also, in 1:1 POPC:DPPC liposomes containing 1 mol %

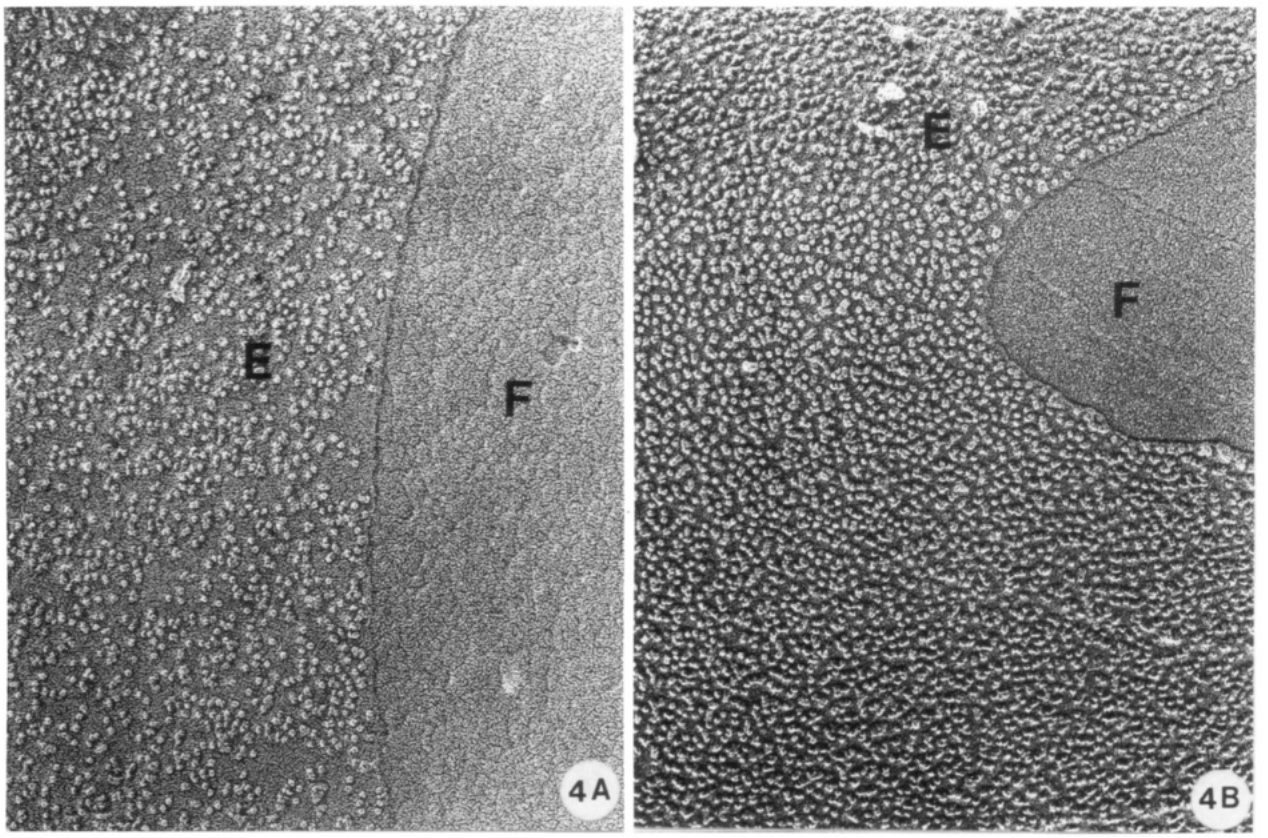

FIGURE 4: Freeze–etch electron micrographs of 1:1 DEPC:DPPC liposomes containing 1 mol % G_{M1} labeled with cholera toxin and quenched from 38 °C (A) and from 4 °C (B). E = external liposomal surface; F = bilayer fracture face. Magnification is 13000×.

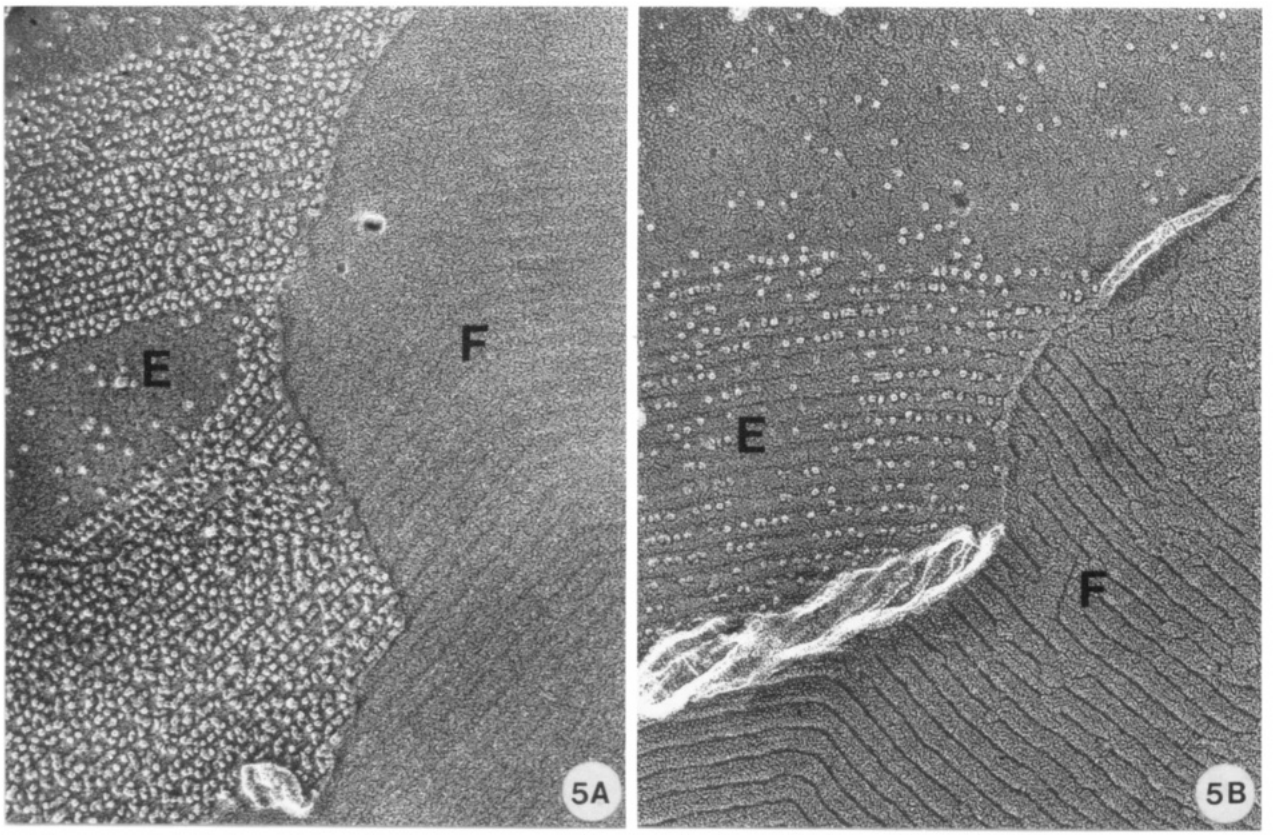

FIGURE 5: Freeze–etch electron micrographs of liposomes consisting of (A) 1:1 POPC:DPPC and (B) 1:1 DMPC:DSPC containing (A) 1.4 and (B) 0.03 mol % G_{M1} labeled with cholera toxin and quenched from 20 °C (A) and from 35 °C (B). The cholera toxin label is predominantly over the ridged regions of the external surface (E). F = bilayer fracture face. Magnification is 130000×.

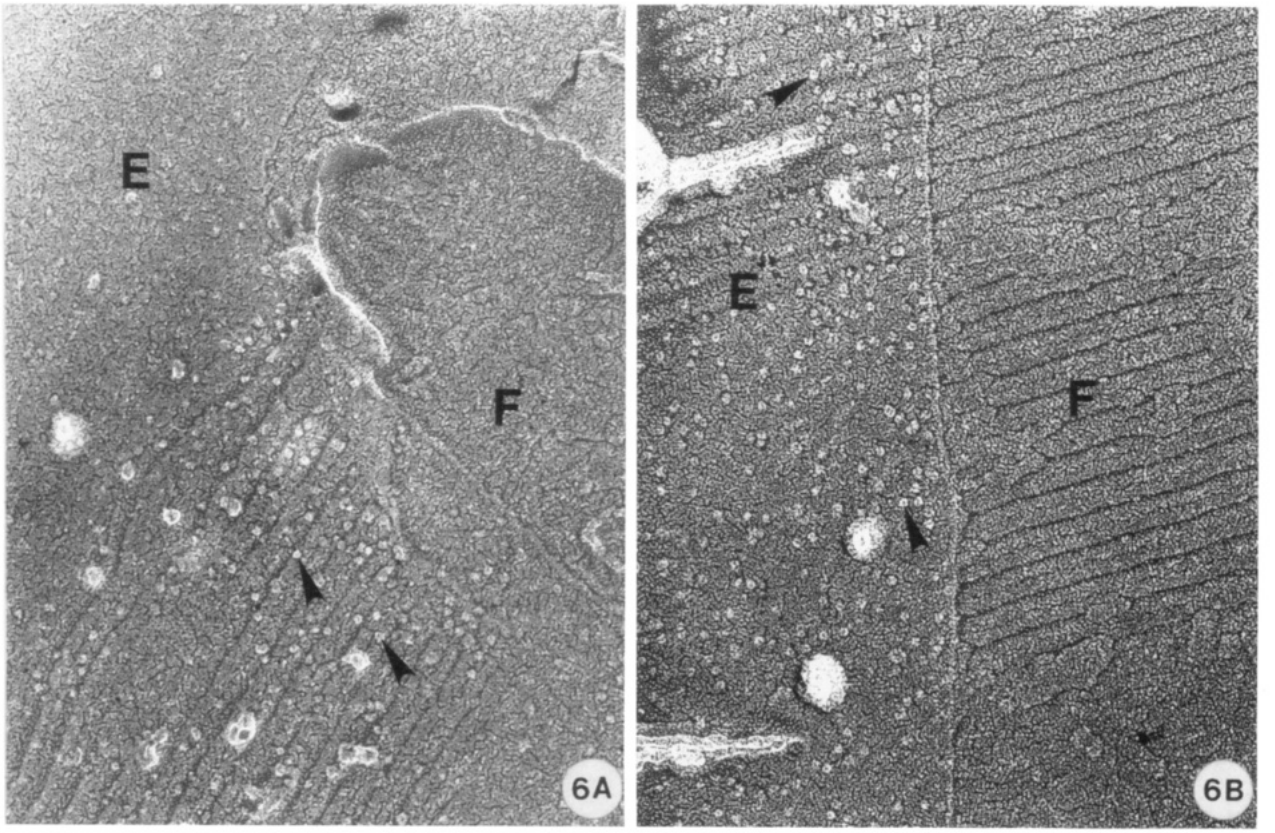

FIGURE 6: Freeze–etch electron micrographs of 1:1 DEPC:DPPC liposomes containing (A) 1 mol % asialo-G_{M1} and (B) 1 mol % Forssman antigen labeled with Fab fragments (arrows) specific for each neutral glycosphingolipid and quenched from 20 °C. The 50-Å Fab fragments are predominantly over the ridged regions of the asialo-G_{M1}-containing liposome (A) and are evenly distributed over ridged and smooth areas of the Forssman-containing liposomes (B). E = external surface of liposome; F = bilayer fracture face. Magnification is 160000×.

Table I: Fatty Acid and Long-Chain Base Analysis of Glycosphingolipids[a]

	G_{M1}	asialo-G_{M1}	Forssman
16:0	2	3	11
18:0	81	80	14
20:0	6	11	12
21:0			3
22:0	9	5	17
23:0			12
24:0			21
24:1			9
C-18 sphingosine	70	70	>95
C-20 sphingosine	30	30	

[a] Values are expressed as weight percent of total and represent the average of two determinations.

Forssman, Fab anti-Forssman label appeared evenly distributed over gel and fluid regions (data not shown). As shown in Table I, Forssman differs from asialo-G_{M1} in its acyl chain composition, which is quite heterogeneous for Forssman. In contrast, G_{M1} and asialo-G_{M1} contain mainly C-18 stearate. In addition, the Forssman sphingoid base is C-18 sphingosine, whereas in G_{M1} and asialo-G_{M1}, C-18 sphingosine accounts for 70% and the remainder is a C-20 base, as noted in Table I.

Discussion

McConnell and co-workers examined bilayers formed from DEPC–DPPC and demonstrated that laterally separated fluid and gel phases can be identified in freeze–etch replicas by the absence and presence, respectively, of the ridged structure characteristic of $P_{\beta'}$ gel phases (Grant et al., 1974). In subsequent papers Peters and Grant (1984) and Peters et al. (1984), using the lectins RCA60 and wheat germ agglutinin as markers, reported that 7 mol % G_{M1}, GD_{1a}, or mixed beef brain gangliosides at 20 °C was found equally distributed in both ridged and smooth regions of liposomes formed from an equimolar mixture of DEPC and DPPC. They did not examine lower concentrations of these gangliosides. In a reexamination of the DEPC–DPPC system, we were surprised to find that G_{M1} is exclusively localized in the gel phase of 1:1 DEPC:DPPC liposomes at 20 °C when it is present at concentrations of 1 mol % or less, although complete surface coverage of both phases by the marker, cholera toxin, occurs at 3.2 mol %. This result was unexpected since molecules added in low concentration to phospholipid bilayers, under conditions where gel and fluid phases coexist, usually partition predominantly into the fluid phase. The subsequent examination of the partitioning of G_{M1} two-phase systems of POPC–DPPC and DMPC–DSPC has shown that G_{M1} also prefers the gel state of these phosphatidylcholines. Preference for the gel state is also indicated by the behavior of G_{M1} at 0.5 mol % in 1:1 DEPC:DPPC liposomes at 38 °C. At this temperature, where the system is in the fluid state but close to the fluidus line on the phase diagram (Figure 2), the cholera toxin label is found in small clusters on the liposomal surfaces. This result suggests that under these conditions G_{M1} tends to phase separate. However, in the same system at 4 °C where the system is entirely gel phase, the cholera toxin marker is uniformly distributed over the entire surface of the liposomes.

The cholera toxin marker used to identify G_{M1} is known to be pentavalent. It is conceivable that cholera toxin binds up to five G_{M1} molecules into a gel-like domain which then associates with the phospholipid gel regions. Thus the segregation of G_{M1} in gel domains might be cholera toxin driven. This situation is very unlikely, at best, for the following reasons. First and foremost is the fact that asialo-G_{M1}, which was derived from G_{M1} by removal of the single sialic acid residue, also prefers gel-phase phospholipid when labeled with a monovalent Fab fragment, whereas Forssman labeled with an Fab fragment does not exhibit a phase preference. Second, the phase structure of the liposomes is formed and annealed before the addition of cholera toxin. Since the lateral mobility of molecules in gel-phase domains is essentially zero on the time scale of the experiment, the inclusion of the putative cholera toxin induced G_{M1} domains within gel regions, as clearly seen in the electron micrographs, would require a global reorganization of the system upon the addition of cholera toxin. Clearly no such reorganization occurs. Third, we have shown in earlier work that, in fluid- and gel-phase, single-component phosphatidylcholine bilayers containing G_{M1}, cholera toxin behaves as a functionally monovalent marker and does not cause aggregation of this glycosphingolipid into patches (Thompson et al., 1985). We therefore think it highly unlikely that the phase preferences of the glycosphingolipids are in any way dictated by the macromolecular markers.

The fact that G_{M1} as well as asialo-G_{M1}, derived from G_{M1} by removal of the negatively charged sialic acid residue, segregates into the gel phase at low concentrations strongly suggests that the polysaccharide moiety of G_{M1} does not play a dominant role in segregation. Clearly the presence or absence of the negative charge on the glycosphingolipid is without effect. In contrast to this result, the neutral Forssman glycosphingolipid does not show a preference for the gel phase. This molecule differs from G_{M1} and asialo-G_{M1} in both the polysaccharide and hydrophobic moieties: Forssman antigen, GalNAcα1$\rightarrow$3GalNAcβ1$\rightarrow$3Galα1$\rightarrow$4Galβ1$\rightarrow$4Glcβ1$\rightarrow$-1Cer; asialo-G_{M1}, produced from G_{M1} by removal of the sialic acid residue, Galβ1$\rightarrow$3GalNAcβ1$\rightarrow$4Galβ1$\rightarrow$4Glcβ1$\rightarrow$1Cer (Kanfer & Hakomori, 1983). As shown in Table I, the ceramide portion of our Forssman preparation contains primarily sphingosine but has a very heterogeneous fatty acyl composition. This is in contrast to both asialo-G_{M1} and G_{M1}, which have a 70:30 ratio of C-18:C-20 sphingoid bases but contain about 80% C-18 acyl chains. If we conclude on the basis of the similarity in gel-phase segregation of G_{M1} and asialo-G_{M1} that the oligosaccharide moiety has no influence on this phenomenon, then the contrast in behavior between these two lipids and Forssman must be due to the differences in the hydrophobic moieties. The predominant difference in this moiety is in the acyl chain composition.

At least two types of molecular interactions between the hydrophobic portions of the glycosphingolipids and the matrix phospholipids may give rise to the observed distribution of glycosphingolipids. The first is interdigitation of methylene chains of molecules in apposing faces of the bilayer. A characteristic feature of all glycosphingolipids is the disparity in length of the two methylene chains in the ceramide portion of the molecule. One chain, contributed by the sphingoid base, is either 18 or 20 carbons in length in mammalian cells. The other methylene chain, contributed by the amide-linked acyl group, varies greatly in length. When the methylene chain length disparity is large, some sphingolipids and certain synthetic phosphatidylcholines have been shown to form interdigitated gel-phase bilayers (Slater & Huang, 1988). It seemed possible that the gel-phase preference shown by G_{M1} and asialo-G_{M1} could be due to the propensity of these lipids to interdigitate in the gel phase. This possibility was ruled out since gel-phase segregation was observed in liposomes in which only the outer surface of the outermost bilayer contained G_{M1}. Interdigitation is, of course, impossible with this asymmetric composition.

The second type of interaction is between methylene chains of neighboring molecules of glycosphingolipid and matrix phosphatidylcholines. Limited calorimetric studies available on neutral glycosphingolipids in aqueous dispersion show phase transition temperatures above 40 °C. These temperatures appear to decrease with increasing numbers of glycosyl residues (Maggio et al., 1985; Curatolo, 1987). For asialo-G_{M1} the transition temperature is 54 °C (Maggio et al., 1985). The value for Forssman is not known. It is possible that the gel-phase preference exhibited by asialo-G_{M1} in two-component, two-phase phosphatidylcholine bilayers reflects the methylene chain length match between asialo-G_{M1} and DPPC or DSPC in the gel phases, which must be rich in these phosphatidylcholines. A match in methylene chain lengths would be expected to maximize van der Waals interactions at a temperature at which asialo-G_{M1}, DPPC, and DSPC individually prefer the gel state. This explanation may also apply to the gel-phase segregation of G_{M1}, although in aqueous dispersions this molecule forms micelles and thus does not exhibit a gel-to-fluid-phase transition. Electrostatic interactions between the negative charges on G_{M1} molecules play an important role in the energetics of micellization. However, when this molecule is present at low concentration in a lamellar phosphatidylcholine bilayer, electrostatic interactions between disperse G_{M1} molecules are minimal. In fact, concentrations in excess of 20 mol % G_{M1} are necessary for the appearance of a coexisting micellar phase (Barenholz et al., 1980; Bertoli et al., 1981). It thus seems quite possible that, at low concentrations of G_{M1} in the two-phase, two-component phosphatidylcholine bilayer, G_{M1} behaves molecularly much like asialo-G_{M1} and exhibits a gel-phase preference for the same reasons as does asialo-G_{M1}.

The Forssman glycosphingolipid preparation is a mixture of at least eight molecular species that span a wide range of acyl chain lengths (Table I). It is possible that Forssman molecules which have acyl chains of about the same length as the higher melting phosphatidylcholine of the matrix pair partition into the gel phase, which must be rich in this component phosphatidylcholine, while Forssman molecules which have acyl chains longer than the phosphatidylcholine matrix molecules remain in the more disordered fluid phase. The preferential partitioning into the fluid phase of Forssman species with longer acyl chains is supported by recent observations of Gardam and Silvius (1989), who found that galactocerebroside and sulfatide containing an amide-linked C-22 fatty acid are completely miscible in fluid DPPC but phase separate in the gel phase of this phosphatidylcholine. Since about 40% of the acyl chains for Forssman are shorter than 22 carbons, if such a differential partitioning of the various Forssman species occurs, the result would be the appearance of a roughly equal distribution of Forssman in both fluid and gel phases. Additional experiments using pure molecular species of Forssman and other glycosphingolipids are needed to assess in detail the influence of the acyl chain and sphingoid base on gel-phase segregation in two-phase, two-component phosphatidylcholine systems.

The external surface of the plasma membranes of mammalian cells has phospholipids and cholesterol as its principal lipid components. The glycosphingolipids are always present in minor amounts. It seems possible to us that any phase structure in the lipid bilayer would be due to the major lipid species. The results of the experiments reported in this paper suggest that if this is the case, the distribution of certain glycosphingolipids such as G_{M1} might be expected to reflect this underlying phase structure, whereas the distribution of other glycosphingolipids might be independent of it.

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